

Mini Review

Beneficial effects of berry fruit polyphenols on neuronal and behavioral aging

Francis C Lau, Barbara Shukitt-Hale and James A Joseph*

Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111, USA

Abstract: It is becoming increasingly clear that although there is a great deal of research being devoted to elucidating the molecular mechanisms involved in aging, practical information on how to forestall or reverse the deleterious effects of aging may be years away. Therefore, it may be beneficial to determine other methods to improve the quality of life in the aging population. A plethora of epidemiological studies have indicated that individuals who consume a diet containing high amounts of fruits and vegetables may have a reduced incidence of age-associated diseases such as cardiovascular or neurodegenerative diseases. Research from our laboratory has suggested that supplementation with fruit or vegetable extracts high in antioxidants can decrease the enhanced vulnerability to oxidative stress and inflammation that occurs in aging, and that these reductions are expressed as improvements in behavior. In addition to research indicating the antioxidant or anti-inflammatory functions of the polyphenolic compounds found in these fruits and vegetables, further studies have suggested that other mechanisms such as cellular signaling may contribute to the beneficial effects of these compounds on aging.

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INTRODUCTION

As populations age in many countries throughout the world, increases in many age-associated diseases such as cancer, cardiovascular and neurodegenerative disease concomitantly occur. One of the most devastating of these diseases is Alzheimer's disease (AD). By the year 2050, for example, 30% of the total population of the United States will be over 65 years of age, and a considerable number of this elderly population will exhibit the most common correlative motor and cognitive behavioral changes that occur in normal aging. Research discussed in this review suggests that the aged brain provides 'fertile ground' for the development of AD and Parkinson's disease (PD), conditions involving even more severe deficits in memory and/or motor function than those seen in normal aging. Unless some means is found to reduce these age-related decrements in neuronal function, health care costs from hospitalization and custodial care will continue to rise exponentially. Thus, in both financial and societal terms it is critical to explore treatments to retard or reverse the neuronal and behavioral deficits that occur in aging. In this review we will describe these age-related motor and cognitive deficits in behavior and show how these deficits are related to increased vulnerability to oxidative stress (OS) and inflammation. We will also describe the possible role of nutritional supplementation with fruits and vegetables containing high amounts of polyphenols,

such as anthocyanins, in forestalling or reversing these deficits.

BEHAVIORAL DECREMENTS

A great deal of research indicates the occurrence of numerous neuronal and behavioral deficits during normal aging. The alterations in memory deficits are seen on cognitive tasks that require the use of spatial learning and memory,¹ while motor function may include decreases in balance, muscle strength and coordination.² Indeed, these changes have been shown in a large number of studies and appear to be expressed both in animals¹ and humans.³ Alterations in memory appear to occur primarily in secondary memory systems and are reflected in the storage of newly acquired information.⁴ Deficits in motor performance are thought to be the result of changes in the striatal dopamine (DA) system, which shows marked neurodegenerative changes with age,⁴ or in the cerebellum, which also shows age-related alterations.⁵

Research shows that the hippocampus mediates place learning, while the prefrontal cortex is critical to acquiring the rules that govern performance in particular tasks (i.e., procedural knowledge). It appears that the dorsomedial striatum regulates spatial orientation involving response and cue learning.⁶ As will be discussed below, substantial research indicates that factors such as oxidative stress⁷

* Correspondence to: James A Joseph, USDA-ARS HNRCA at Tufts University, 711 Washington street, Boston, MA 02111, USA

E-mail: james.joseph@tufts.edu

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and inflammation⁸ are major contributors to the behavioral decrements seen in aging. As is well known, cognitive deficits, along with markers of oxidative and inflammatory stressors, are magnified in dementing diseases such as AD.⁹

OXIDATIVE STRESS

Reactive oxygen species (ROS) collectively refer to oxygen radicals and non-radicals that are readily converted to radicals^{10–12} which are by-products of normal aerobic metabolism.^{13,14} The production of ROS is normally counterbalanced by cellular defense systems,^{15,16} but approximately 1% of the ROS escape daily elimination to produce oxidative cellular damage leading to increased oxidative stress.¹⁷

The brain is especially susceptible to oxidative stress^{16,18} for the following reasons: (a) the brain has relatively ineffective antioxidant defenses that include very low levels of catalase activity and only moderate amounts of the endogenous antioxidant enzymes, superoxide dismutase and glutathione peroxidase; (b) although comprising approximately 2% of the body mass, the brain utilizes 20% of the total oxygen consumption and is enriched with readily peroxidizable polyunsaturated fatty acids; (c) the brain has high levels of iron and ascorbate, which are the key catalysts for lipid peroxidation; (d) many neurotransmitters themselves are subject to autooxidation to generate ROS;^{19–22} and (e) finally, except for those in some restricted regions of the brain, neuronal cells are post-mitotic and tend to accumulate oxidative damage.^{13,23,24} Furthermore, as the brain ages, all of these susceptibilities contribute to the increased vulnerability of the brain to OS during aging,^{25–29} as well as the pathogenesis of age-related neurodegenerative diseases such as AD, PD, and amyotrophic lateral sclerosis (ALS).^{30–32}

INFLAMMATION

Evidence suggests that increased inflammation in the central nervous system is associated with normal aging.^{33–36} By middle age there is an increased glial fibrillary acidic protein expression³⁶ that later, in the elderly, even occurs in the absence of an inflammatory stimulus.³⁷ Research also indicates that tumor necrosis factor- α (TNF α) is produced in higher amounts during cytotoxic reactions in the elderly.³⁸

In turn, increased inflammation is associated with enhanced ROS production.^{39–42} Over-production of ROS has been reported in several inflammatory diseases^{43–45} and is associated with microglial cell activation^{44,46–49} as well as the activation of inflammatory mediators such as the inducible nitric oxide synthase (iNOS), interleukin-1 β (IL-1 β), TNF α , and nuclear factor kappa B (NF- κ B).^{50–57}

Importantly, oxidative stress-mediated inflammation has also been attributed to neurodegenerative disorders including AD and PD,^{58–63} and the increases

in sensitivity of the brain to OS and inflammation in senescence may be the cause of the behavioral deficits outlined above.

NEUROPROTECTIVE EFFECTS OF FRUIT POLYPHENOLS

The key, then, to possibly reducing the incidence of age-related dementing diseases might be to alter the neuronal environment such that neuroinflammation and oxidative stress and the vulnerability to them are reduced. Research from our laboratory and others suggests that the combinations of antioxidant/anti-inflammatory polyphenolics found in fruits and vegetables may be effective in this regard.

Plants, including food plants (fruits and vegetables), synthesize a vast array of secondary chemical compounds that, while not involved in their primary metabolism, are important in serving a variety of ecological functions that enhance the plant's survivability. Interestingly, these antioxidant/anti-inflammatory compounds may be responsible for the multitude of beneficial effects that have been reported for fruits and vegetables on an array of health-related bioactivities. However, up until very recently the majority of the dietary agents employed to alter behavioral and neuronal effects with aging included such nutritional supplements as vitamins C or E, and garlic,⁶⁴ with only limited attention given to the possible value of polyphenolic-containing plant-derived supplements such as ginseng, *Ginkgo biloba*, and ding lang.⁶⁵

In an exploration of the effects of plant-derived polyphenols in aging, we assessed whether fruit or vegetable intervention would reverse cognitive and motor behavioral deficits in aged animals. We found that dietary supplementation (for 8 weeks) with spinach, strawberry or blueberry (BB) extracts in an AIN-93 diet was effective in reversing age-related deficits in neuronal and cognitive function as shown by Morris water maze (MWM) performance in aged (19 months) F344 rats.⁶⁶ However, only the BB-supplemented group exhibited improved performance on tests of motor function. Specifically, the BB-supplemented group displayed improved performance on two motor tests which rely on balance and coordination – rod walking and the accelerating rotarod – while none of the other supplemented groups differed from control on these tasks.

In the same study, the rodents in all diet groups, but not the control group, showed improved working memory (short-term memory) performance in the MWM, demonstrated as one-trial learning following the 10 min retention interval.⁶⁶ We also observed significant increases in several indices of neuronal signaling (e.g., muscarinic receptor sensitivity), and found that the BB-supplemented diet reversed age-related deregulation in calcium-45 buffering capacity, an important index of neuronal dysfunction in aging.⁶⁶

The fruits and vegetables used in these studies were all high in antioxidant capacities as measured by the

modified oxygen radical absorbance capacity (ORAC) assay.⁶⁷ While this may provide some explanation for the positive benefits obtained, assessments of oxidative stress in the tissue from these animals revealed only modest decreases. Examinations of ROS production in the brain tissue obtained from animals in the various diet groups indicated that the tissue (e.g., striata) obtained from all of the supplemented groups exhibited significantly less ROS levels than the controls via 2',7'-dichlorofluorescein diacetate (DCF) assay. However, these decreases did not appear to be sufficient to account for the observed significant beneficial effects of BB supplementation on motor and cognitive function. It was clear from this⁶⁶ and a subsequent⁶⁸ study that the significant effects of BB on both motor and cognitive behavior were due to a multiplicity of actions, in addition to those involving antioxidant and anti-inflammatory activities.

In another study using APP/PS1 (amyloid precursor protein/presenilin-1) transgenic mice maintained on a blueberry-supplemented diet, we found that extracellular signal regulated kinase (ERK) and protein kinase C (PKC), two important transcription factors in learning and memory, were upregulated. These findings indicated that the polyphenols found in the berry fruit may affect neuronal signaling.⁶⁹ These studies also suggested that BB supplementation may actually prevent cognitive deficits, as shown by enhanced performance in a Y maze, directly enhancing neuronal signaling, and offsetting any putative deleterious effects of the amyloid deposition. The data also revealed that blueberry supplements seemed to enhance signaling at the level of the kinases and, more directly, to increase the sensitivity of muscarinic receptors as indicated by increased striatal, carbachol-stimulated GTPase activity.⁶⁹

We are currently exploring additional mechanisms involving these effects, and recent research from our laboratory using a COS-7 cell model indicates that BB polyphenolics can directly alter oxidative stress signaling,⁷⁰ particularly with respect to MAP kinase (e.g., ERK) activation and cyclic AMP response element binding protein (CREB).⁷¹

These findings, combined with additional preliminary research showing that BB supplementation in senescent rats may increase neurogenesis,⁷² suggest that at least part of the efficacy of BB supplementation may be to allow aging brain regions involved in both motor and memory performance to communicate more effectively with each other via the formation of new neurons. Thus, BB supplementation, by facilitating both neurogenesis and enhanced neuronal signaling, may strengthen areas of the brain showing the ravages of time.

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